CHAMBERS' MICROMANIPULATOR FOR THE ISOLATION OF A SINGLE BACTERIUM

MORTON C. KAHN

From the Department of Hygiene, Cornell University Medical College, New York City

As I have been working for several years on the isolation of bacteria with Barber's apparatus and have recently been using Chambers' instrument for the same purpose, Dr. Chambers suggested that I make some comments on the relative merits of the two instruments together with a short review of Barber's isolation method.

The mechanical principles involved in the construction of this instrument are entirely different from those of Barber's pipet holder, while the basic methods of manipulation are essentially the same. There are several noteworthy features in the Chambers' instrument which modify and improve the original Barber technic. These features may be best described under two headings: (1) advantages in construction, and (2) advantages from the use of various accessories to aid in manipulation.

ADVANTAGES IN CONSTRUCTION

The mechanical principles on which the instrument is based are fully described in the preceding article by Chambers.[1] The absence of parts which may loosen by wear and tear renders possible great precision in the manipulations. While excellent work may be done with the Barber pipet holder, the parts wear somewhat after several months' use, giving rise to a certain amount of false motion. For instance, when one desires to move the pipet laterally one may encounter an unexpected vertical motion. The Chambers apparatus used by me had been in use for two years, and in spite of this I was unable to detect any false motion.

A second advantage is that the instrument clamps directly on the stage of the microscope giving much greater rigidity than is possible with the metal flange which has to be attached to the stage of the microscope when Barber's instrument is used. Third, the smaller size of the instrument brings all manipulations closer to the microscope and

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[1] Jour. Infect. Dis., 1922, 31, p. 334.

eliminates accidental jostling of the pipet holder which may shift the needle out of focus. This compactness also makes it feasible to use a short pipet which is easier to focus and is in less danger of contamination as less of the pipet is exposed.

ADVANTAGES OF THE ACCESSORIES IN CHAMBERS' APPARATUS

Barber's instrument possesses no accessories so that all the adjustments, both preliminary and operative, have to be made by means of the same finely threaded screws with a consequent waste of considerable time.

In Chambers' instrument the several accessories are as follows:

1. There is a brass collar (fig. 2′) through which the shank of the pipet is inserted before clamping it in the pipet carrier of the instrument. Besides insuring rigidity to the pipet and thus greater accuracy for manipulation, the collar facilitates bringing the pipet into the field of the microscope. It also steadies the pipet as it is being withdrawn from the moist chamber, thus minimizing contamination or injury to the delicate tip.

2. For the vertical manipulation of the pipet there are three different adjusting devices: first, the telescoping pillar for roughly adjusting the pipet to the height of the moist chamber, after which it may be tightly clamped; second, another coarse adjustment operated by a spring screw with which one may bring the pipet into focus, and, third, the fine adjustment of the knurl headed screw (fig. 1), which is used in the actual operation of isolation. The first two devices are for the coarse adjustment and enable one to use moist chambers of practically any height. They aid greatly in the technic of the vertical adjustment, which is the most important one from the bacteriologic point of view.

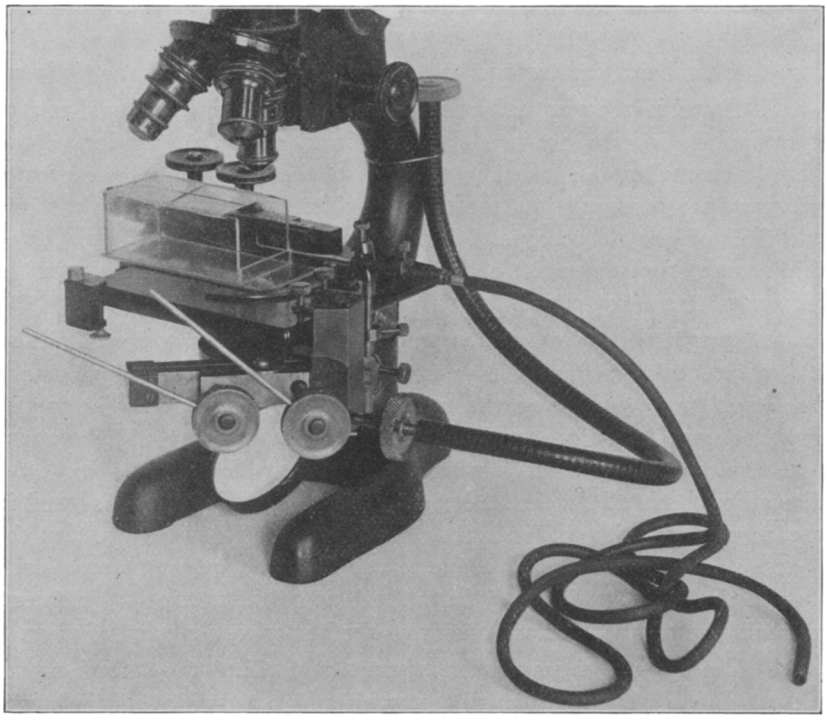
3. The use of levers on the screws controlling the lateral movements insures great delicacy to the touch and is a decided aid in bringing the pipet directly under the droplet containing the organism, especially when working with the higher power or oil immersion lens where the slightest movement is greatly magnified.

4. The flexible shaft attached to the vertical control screw brings the movement of that screw behind the microscope away from all working parts and close to the fine adjustment of the microscope.

For bacteriologic work it is more convenient to have the moist chamber as Barber originally devised it, viz., with its open end so placed that the pipet may project into it from the left side. This

facilitates the frequent interchange of pipets so necessary in isolating bacteria. For this purpose it is necessary to have the pipet holder attached to the left side of the microscope. This can be done with the form which Chambers designates as right-handed by fastening it on the left side of the microscope stage near the outer corner (fig. 1).

In the preceding article it has been recommended to have the height of the moist chamber equal to the working focal distance of the sub-



Micromanipulator mounted on the left side of the microscope for isolating bacteria. Note Barber's moist chamber with the coverslip marked with cross lines to aid in locating areas. The chamber shown here is higher than necessary.

stage condensor. This limits the height to one which may make the chamber too shallow to be convenient for frequent interchanges of the pipet. I, therefore, use a chamber ¾ of an inch high, 1 inch wide and 2½ inches long. It will be noted that this moist chamber is not only deeper but also wider than the one Chambers uses for cytological work.

THE ISOLATION METHOD

For the isolation of a single bacterium one must have the under surface of the coverslip so treated as to hold minute droplets without fear of their spreading or possibly running together. The droplets placed on the coverslip must be slightly hemispheroidal in order that their outlines may be distinct and all parts of it clearly visible. Also, in order to maintain these droplets throughout the operation, the moisture conditions within the chamber must be sufficient to prevent their evaporation and at the same time must not be too great for fear of flooding them.

It is necessary, therefore, to have the surface of the coverslip specially prepared. Barber, after smearing the cleaned coverslips with petrolatum, washes them with soap and water to get rid of the excess of petrolatum. The coverslips are then carefully cleaned with a dry cloth, heated enough to soften the petrolatum and rubbed again while still warm. The aim is to remove as much petrolatum as possible without the use of excessive heat or any fat dissolving reagent other than soap. If an excess of petrolatum is left on the cover, small particles will appear in the droplets and may be mistaken for bacteria. If all petrolatum is removed, the droplets run together and make successful isolation impossible. Instead of soap and water one may use 95% alcohol with equally good results. One must realize that success or failure in isolation work depends on a proper treatment of the coverglass.

The method of procedure for the isolation of a bacterium may be summarized as follows: [2]

1. Prepare a young liquid culture from a subculture not more than 18 hours old.
2. Insert the tip of a needle into a tube of the liquid culture and convert the needle into a pipet by gently rubbing it against the wall of the tube. Then with a rubber tube on its shank suck up a small amount of the culture.
3. Insert the pipet in the brass collar, then clamp it in the pipet holder of the instrument and bring the tip into focus in the center of the microscopic field (see figure). Raise the pipet until its tip touches the undersurface of the coverslip and expel an appreciable droplet. This may have to be diluted with sterile fluid if the culture is too dense.
4. After securing a moderately dilute preparation fill the same or a new pipet to a little below its bend. Lower the pipet, and with the aid of the mechanical stage, bring another portion of the coverslip into view. By alternately raising and lowering the pipet a series of minute droplets will be produced on the coverslip wherever the pipet touches it. The fluid runs out by capillary attraction and needs no blowing. Some of these droplets will be found to contain a single micro-organism.

[2] The method of making the pipets is described in the preceding article by Chambers.

5. Replace this pipet with a new sterile one containing a small amount of sterile liquid medium which must not run below the elbow. This new pipet is now brought directly under a droplet containing a single micro-organism. The pipet is then slowly raised and as soon as it touches the surface the droplet with the contained organism will flow into it. This occurs by capillary attraction and no suction is required.

6. This pipet, which is known to contain only one micro-organism, is carefully removed from the apparatus and its tip inserted into a tube containing a suitable sterile medium. The entire contents of the pipet are now to be expelled by blowing. As an added precaution it is well to break off the tip of the pipet in the culture medium. The blowing may be done by mouth or by a rubber bulb operated either by the hand or the foot.